ON THE OCCURRENCE OF HEMOLYTIC STREPTOCOCCI IN THE STOOLS OF SCARLET FEVER

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This work is one step in a study of the mechanism of infection from the gastro-intestinal tract. Most of the bacteria swallowed are believed to be killed in the acid stomach contents but it is possible that pathogenic bacteria may reach the intestines enmeshed in food particles or washed through with water. In order to establish the relationship of the bacteria in the intestines to foci of infection it is first necessary to know whether pathogenic bacteria are present in the intestines, how frequently, and in how great numbers. Hemolytic streptococci have been chosen because of their frequent occurrence in the mouth, ease of identification, and possible relationship to certain infectious processes. As hemolytic streptococci occur in great numbers in the throats of scarlet fever patients, a systematic study of the stools in scarlet fever was undertaken to determine the presence or absence of these organisms.

There is little in the literature with regard to studies of hemolytic streptococci in the feces. Broadhurst [1] isolated 9 strains of S. infrequens from 31 stools; Holman [2] reports 9 strains of S. infrequens Broadhurst and 4 strains of S. pyogenes, a total of 13 of 53. Oppenheim [3] found hemolytic streptococci in 5 stools from 15 normal individuals; and D. J. Davis [4] was unable to isolate any from the stools of 53 patients, 4 of whom had scarlet fever. Baermann and Eckersdorff [5] in a study of dysentery stools found streptococci which on blood agar were said to be definitely hemolytic. Winslow and Palmer,[6] Fuller and Armstrong,[7] and others have studied the fermentation reactions of fecal streptococci but they make no mention of growth of these organisms on blood agar.

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[1] Jour. Infect. Dis., 1915, 17, p. 277.
[2] Jour. Med. Research, 1916, 34, p. 377.
[3] Jour. Infect. Dis., 1920, 26, p. 117.
[4] Jour. Infect. Dis., 1920, 26, p. 171.
[5] München. med. Wchnschr., 1909, 56, p. 1169.
[6] Jour. Infect. Dis., 1910, 7, p. 1.
[7] Jour. Infect. Dis., 1913, 13, p. 442.

METHODS

Specimens were obtained from scarlet fever patients in the Durand Hospital. A portion of the stool was placed in a small sterile jar and examined from 1 to 48 hours later. When possible the specimen for examination was taken from the inside of the stool, and placed in about 3 c c of normal salt solution, making a moderately dense suspension; 2 or 3 loopfuls of this suspension were placed in another tube of salt solution, mixed, and with a small pipet about 0.25 c c was transferred to a third tube, mixed, and a similar amount to a fourth, making 3 dilutions. It was estimated that, using a wire loop 4 mm. in diameter, from 40 to 150 mg. of a soft-formed stool were used in making the first suspension. Dilutions were made with a 2 mm. loop. The water content of the stool is so variable that an exact estimate of the amount of feces obtained is impossible. Surface inoculations on plates were made, and also plates were poured, using 10% goat blood agar. After 24 hours' incubation the colonies with hemolysis were transplanted to blood or plain agar and microscopic examination made. Bile solubility, virulence, fermentation, agglutination and opsonic tests were made with various strains isolated from the feces.

RESULTS OBTAINED FROM REPEATED STOOL EXAMINATIONS FROM INDIVIDUAL PATIENTS

	Number of Stools Examined	Number of Stools from Which Hemolytic Streptococci Were Isolated
1.	26	2
2.	22	4
3.	12	0
4.	10	1
5.	10	0
6.	9	3
7.	5	2

The total number of stools examined was 309, in 37 of which (12%) hemolytic streptococci were isolated. The stools were collected from 85 different patients, all with scarlet fever, and streptococci were isolated from 26 (30%). From 31 patients only one stool examination was made, of which 6 (19%) showed hemolytic streptococci. To 11 patients a cathartic was given and several stools obtained the same day. In 3 instances (18%) hemolytic streptococci were isolated.

The number of colonies of hemolytic streptococci on the plates on which they were noted was variable, but usually there were very few in comparison to the other organisms. On a blood-agar plate con-

taining some 200 or 300 colonies, usually 6 to 10 were definitely hemolytic streptococci. Occasionally on very thickly seeded plates with innumerable colonies only 3 or 4 hemolytic colonies were observed.

The age of the patients varied from 20 months to 45 years. Specimens of stools were examined at intervals of 1 to 62 days from the onset of the disease. In 2 instances hemolytic streptococci were isolated from watery stools, 13 from semisolid, 3 from soft formed, and 2 from hard; the greater number of stools examined were semisolid. There was no apparent relationship between the occurrence of hemolytic streptococci in the stools and the age of the patient, duration of the disease, or character of the stool. Stools obtained after the administration of a cathartic yielded a greater proportion of green-producing streptococci but the proportion of hemolytic streptococci was not increased.

On blood-agar surface streaked plates the hemolytic streptococci isolated grew, after 24 hours' incubation, as small, round, gray, somewhat raised colonies with a clear zone of hemolysis about 2 mm. in diameter, and a hazy border. The deep colonies under the microscope were small, biconvex or oval, with no red blood corpuscles visible for a diameter of 1 to 2 mm. On plain agar after 24 hours' incubation a small gram-positive coccus in chains was seen. All strains were insoluble in bile.

Fermentation tests were made with 22 strains of hemolytic streptococci isolated from the feces. These strains were grown 7 days in 1% lactose, mannite, salicin, and inulin; all fermented lactose and salicin but not mannite nor inulin, corresponding to Streptococcus pyogenes of Holman.[2]

Using 0.5 c c of an 18-hour broth culture, 20 strains were injected intraperitoneally into as many white mice. Of these, 13 died within 24 hours and hemolytic streptococci were isolated from the heart blood and peritoneal fluid; 4 of these strains, causing death of white mice in 24 hours were opsonified or agglutinated by the serum of a sheep injected with hemolytic streptococcus from scarlet fever.[8] One strain that killed a mouse was neither opsonified nor agglutinated by this serum and 2 other strains not killing mice were neither opsonified nor agglutinated.

Agglutination and opsonic tests were made using blood from a sheep injected with hemolytic streptococci isolated from the throat of

[8] Tunnicliff, R.: Jour. Am. Med. Assn., 74, p. 1386.

a scarlet fever patient before the appearance of the rash. In all, 11 strains were tested for opsonic and agglutinative reactions; of these, 6 strains were either agglutinated or opsonified by the immune serum. With two strains the points of opsonic extinction were 1:300 and 1:150, and each of the strains was agglutinated in a dilution of 1:200; 2 strains gave points of opsonic extinction of 1:150 and 1:90, but the determination of agglutination was unsatisfactory by reason of spontaneous agglutination in the normal sheep serum controls; 3 strains failed to give any opsonic or agglutinative reaction; 2 strains gave a positive opsonic reaction in a dilution of 1:15, and 2 a negative reaction in the same dilution; these 4 strains were not agglutinated by the immune serum. It is of interest to note that one of the strains that did not react was isolated from a patient who had many hemolytic streptococci in the throat which also failed to react with the immune serum. There was some question clinically as to whether this patient had scarlet fever.

At intervals throat swabs from patients were streaked on blood agar plates and hemolytic streptococci found at one time or another in all patients examined. These organisms on blood agar appeared exactly similar to those isolated from the stools, and fermented lactose and salicin, but not mannite.

Green-producing streptococci were frequently noted and some fermentation tests were made: 6 strains fermented lactose and salicin, 2 strains fermented lactose, salicin and mannite, Streptococcus mitis and fecalis, respectively. Colon bacilli were, of course, encountered in great numbers; a large proportion were definitely hemolytic, a fact which caused difficulty in their differentiation from streptococci. There was a greater similarity between the deep colonies of hemolytic streptococci and colon bacilli than between those on the surface.

SUMMARY

Typical hemolytic streptococci were isolated from the feces of 30% of 85 scarlet fever patients. They occurred in the feces at irregular intervals with no definite relation to the age of the patient, character of the stool, duration or intensity of the illness. The number of colonies was small in comparison to the number of other organisms, such as B. coli, staphylococci, and green-producing streptococci, but when it is considered that only a small part of one loopful of the stool was studied it seems probable that hemolytic streptococci occur

in the stools of scarlet fever patients perhaps more frequently than is indicated by these figures. Six of 11 strains of hemolytic streptococci isolated from the stools of scarlet fever patients were agglutinated or opsonified by immune serum from a sheep injected with hemolytic streptococci obtained from the throat of an early case of scarlet fever.[8]

[8] Tunnicliff, R.: Jour. Am. Med. Assn., 74, p. 1386.